# PHYSIOLOGY OF FLOWERING IN
# CUPRESSUS ARIZONICA GREENE SEEDLINGS

By

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A DISSERTATION PRESENTED TO THE GRADUATE
COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

# ACKNOWLEDGMENTS

This work was done with the support of a research assistantship provided by the School of Forest Resources and Conservation and the Agricultural Experiment Station, Institute of Food and Agricultural Sciences. Financial support also was provided by International Programs. This support is gratefully acknowledged.

The author wishes to thank Dr. R. G. Stanley, Chairman of the Thesis Committee, Dr. J. R. Edwardson, Dr. P. L. Pfahler, Dr. R. E. Goddard and Dr. I. K. Vasil, members of the Thesis Committee for moral support and help. My gratitude, in particular, is expressed to Dr. C. A. Hollis for encouragement, critical review and help. The assistance of Miss Bonita K. Carson with typing is truly appreciated. The encouragement, help and patience of my wife, Lujan, are especially acknowledged.

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Abstract of Dissertation Presented to the
Graduate Council of the University of Florida in Partial
Fulfillment of the Requirements for the Degree of Doctor of Philosophy

# PHYSIOLOGY OF FLOWERING IN *CUPRESSUS ARIZONICA* GREENE SEEDLINGS

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August, 1973

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Experiments were undertaken to test the influence of reproductive development in *Cupressus arizonica* on height growth. The influence of fertilizer, urea, auxin, gibberellin (GA) concentration and photoperiod on megasporangiate and microsporangiate strobili induction was tested. Studies were made on the effect of GA applied locally to different parts of the seedling on the production of megasporangiate and microsporangiate strobili. The direction which the strobili inducing stimulus moved, and height growth were studied. To gain some insight into the physiological changes, several enzyme patterns were followed as the seedlings underwent the transition to flowering.

Under the environmental conditions used in this study, GA applications to seedlings of *Cupressus arizonica* increased height. Height of some GA-treated plants not producing strobili was compared to those plants which did produce strobili. No growth suppression was found in flowering plants. Fertilizer application enhanced the seedling response to GA in vegetative and reproductive development.

Auxin or urea did not increase induction of megasporangiate strobili, but a combination of auxin and GA promoted vegetative growth, microsporangiate and megasporangiate strobili induction. The best method to produce large numbers of megasporangiate strobili consisted of spraying the top part of the plants with a high GA concentration under long days. The flowering stimulus has an acropetal movement. Reasons for this are probably related to the nutritional status of the plant, GA affect on metabolic nutrient sinks, and GA movement in plants.

Isozyme patterns of enzymes, α-amylase, peroxidase, and polyphenol oxidase were studied by polyacrylamide gel electrophoresis. Total protein patterns were also studied by this technique. Total activity of phenylalanine ammonia-lyase in induced and noninduced buds was also evaluated. By this technique, no changes were detected which could be related to the transition to flowering, some enzyme changes in α-amylase which were observed probably reflected modified environment, i.e. temperature influences on seedlings. These results suggest that the subcellular metabolic changes preceeding flower induction in *Cupressus arizonica* occur at a level of magnitude, in different cell fractions, or in a quantity of tissue, below the limits of analysis used in this study.

# Chapter 1

## GENERAL REVIEW

### Introduction

The reproductive and vegetative development processes in trees regulate seed and fiber production. Understanding the inherent control mechanisms of these processes is important from both a scientific and commercial standpoint. The study of physiological changes occurring within a tree during transition to flowering may elucidate some of the internal control mechanisms and facilitate regulation of tree growth.

In Pinaceae, chemical induction to reproductive primordia has not yet been possible. However, several genera in the Taxodiaceae and Cupressaceae have been found to respond to applications of gibberellins (GA) by initiating strobili in less than one-year-old seedlings. Normally these plants only produce strobili and seed beyond the age of four or five years. These species are useful in the laboratory to study the internal mechanisms leading to flowering. They also lend themselves to the study of the influence of chemical and fertilizer treatments on seedlings with active strobili development, and to the study of factors influencing reproductive development or vegetative growth.

Extensive studies have been carried out in *Cryptomeria japonica* and *Cupressus arizonica* on the effect of photoperiod and concentration of applied GA, studies of the endogenous growth substances have also been reported (Hashizume, 1961c, 1969). But no study has been reported on the influence of strobili development on vegetative growth and of the enzyme changes occurring after GA induction of strobili.

The purposes of this study were to increase our understanding of the physiological and biochemical mechanisms affecting flowering in *Cupressus arizonica* after GA application. Attempts were made to discern the effect of reproductive development on vegetative growth, to increase the production of megasporangiate in relation to microsporangiate strobili, to verify the effect of localized external applications of GA, and, to study the changes in isozymes with flowering.

## Background Literature

### Flower Induction in Angiosperms

The historical aspects of major events in flower induction have been extensively reviewed (Lang, 1965, Chailakhyan, 1968, Evans, 1971, van de Pol, 1972). Tournois (1912) first reported the effect of short day length on flowering, Garner and Allard (1920) confirmed this, and applied the terms photoperiod, photoperiodism and critical photoperiodism. The "florigen" concept (Chailakhyan, 1936) as the substance responsible for flowering is widely questioned (Evans, 1971). Supporting evidence from grafting experiments with 35 different species shows that the flowering stimulus is transmitted from induced plants to noninduced ones (Chailakhyan, 1968). Although florigen has not been isolated the possibility of its existence is still recognized (Chailakhyan, 1968, van de Pol, 1972). The flowering stimulus can be characterized internally by changes in relative amounts of growth substances, changes in the kinds of RNA, changes in enzymes and other metabolites.

Photoperiod, which determines flowering in many plants, is detected by the leaves of the plants, and acts through the intermediary compound, phytochrome. In the case of the flowering stimuli, we do not know exactly how they are translated to differentiate flowers. Steps from the light quality perception by phytochrome to flower formation also are not well known.

GA promotes flowering in several long day plants under noninductive conditions. It also evokes several other responses. Recent reviews describe many effects of GA on plants (Galston and Davies, 1970, Wareing and Phillips, 1970). These include increase in cell permeability (Artamonova and Artamonov, 1971), preventing destruction of indoleacetic acid by indoleacetic acid oxidase (Kögl and Elema, 1960) and channelling a nutrient supply to GA treated parts (Wertheim, 1971, van Overbeek, 1966). The nutrients accumulated are mainly carbohydrates (Saniewski and Pieniazek, 1972).

Carbohydrate accumulation has often been related to flowering (Sachs and Hackett, 1969, Henrickson, 1954, Cumming, 1967, Davis, 1957, Stanley, 1958). The carbohydrates may accumulate due to flow from other plant parts, or due to starch degradation indirectly caused by GA. In barley seed aleurone cells, GA released by the embryo, or supplied exogenously, causes the production of a new mRNA which in turn is translated into new α-amylase (Varner and Chandra, 1964). This enzyme acts on the starchy endosperm, releasing sugars necessary for embryo development. *De novo* production of enzyme is one consequence of GA application to plants.

The alteration of the meristem toward development of reproductive buds in plants requires a *de novo* synthesis of new proteins. Such new proteins were detected in meristems undergoing differentiation to flowering (Barber and Steward, 1968), and also in developed flowers

(Marushige and Marushige, 1962; Tran and Trippi, 1969, Kahlem, 1970) Several studies support the view that transition to flowering is provoked by *de novo* DNA transcription and translation (Salisbury, 1963, Searle, 1965; Bernier, 1971). Hess (1959) found that plants subjected to a flower inducing photoperiod, synthesize new RNA. If RNA synthesis is blocked by thiouracil, the plant remains vegetative, suggesting that transcription is necessary for production of enzymes involved in flower development. For vegetative development, no new RNA was required. Other studies suggest that no new transcription is needed for flowering, but new translation is needed (Heslop-Harrison and Heslop-Harrison, 1970, Cherry, 1970). Thus, the flowering stimulus would initiate production of new enzymes. It has also been suggested from studies with angiosperms that GA acts first on a cytoplasmic factor which in turn would derepress DNA (Wareing and Phillips, 1970).

### Flower Induction in Conifers

Conifers usually flower abundantly only after attaining adult age. Most have their microsporangiate strobili initiated in summer under long days, and megasporangiate strobili initiated about two weeks later (Mergen and Koerting, 1957; Goo, 1961). Artificial control of flower induction is difficult in most conifers, and fertilization, girdling, strangulation, root pruning, crown release and thinning have been attempted. Many such treatments increased seed production, but no precise method worked for all conifers, as, say, photoperiod in some angiosperms, now were the effects sustained.

Conifers require different internal and external conditions for induction of megasporophyllic and microsporophyllic strobili. Giertych (1967) suggests that microsporangiate strobili are induced as long day plants and megasporangiate strobili as short day plants in pines. Physiological maturation and nutritional status of the tree are important for the kinds of strobili developed. Aging is correlated with microsporangiate development, and maturation with megasporangiate development (Moorby and Wareing, 1963). Maturation is dependent on a good nutritional status, while aging is not. Thus, growth suppression of trees leads to microsporangiate strobili, and heavy fertilization to megasporangiate strobili production (Mergen, 1961). The size and physiological status is more important for reproductive development than the number of growth cycles a tree has passed (Wareing, 1959; Ebell and McMullen, 1970; Allsopp, 1967).

Microsporangiate strobili develop at the lower portion of the tree, and megasporangiate strobili develop at the upper part (Hard, 1964). This differential positioning of reproductive structures may be due to variations in light and atmospheric conditions, distribution of nutrients and differential concentrations of growth substances. Development of microsporangiate strobili is more sensitive to 2, 4-D than development of megasporangiate strobili (Brown and Greene, 1961, Hong, 1963). An early flushing date favors megasporangia development, while late flushing favors microsporangiate strobili formation (Larson, 1961). Defoliation of *Pinus thunbergii* caused a transition of microsporangia primordia to megasporangia (Hashizume, 1961a). The same effect is obtained by covering the buds with waxed paper or applying heavy nitrogen fertilization (Hashizume, 1961b). Cuttings of *Pinus radiata*, maintained in culture for two years, under nitrogen deficiency produce only microsporangiate strobili, while trees with heavy nitrogen fertilization produced almost exclusively megasporangiate strobili (Sweet and Will, 1965).

Grafting of young seedlings on mature trees promotes development of microsporangiate strobili on the seedlings (Mirov, 1951). Debudding *Pinus sylvestris* lateral shoots in winter leads to heavy microsporangiate strobili formation (Wareing, 1952) but if done in conjunction with removal of the leader shoot, no increase in strobili production is detected (Melchior and Heitmüller, 1961).

Summarizing, the data suggest that the development of mega- and microsporangiate strobili is differentially influenced by the nutrient status of the plant, by growth regulators, position in the tree and metabolic activity of the bud. Manipulation of one or more of these conditions results in still unrecognized internal metabolic shifts, which can influence the reproductive capacity of the tree.

### GA Effect on Conifers

Most conifers tested for growth response to GA did not show the expected growth acceleration observed in angiosperm seedlings (Melchior, 1961). Then Kato in 1958 reported that *Cryptomeria japonica* would flower after GA applications. This work was expanded to show that low concentration of GA favored induction of microsporangiate strobili and high concentrations of GA or late summer application induced megasporangiate strobili (Hashizume, 1959).

Using the same induction method with *Cupressus arizonica*, Pharis produced only microsporangiate strobili on seedlings as young as 55 days old (Pharis et al., 1965a). No effect on vegetative growth was observed after the GA treatment. Concentrations of GA applied ranged from 0.1 to 1000 ppm; they all induced strobili, although the strobili induced at the lower GA concentration failed to mature. No megasporangiate strobili were observed. Several other Cupressaceae and Taxodiaceae responded in a similar fashion to GA applications (Pharis and Morf 1967, 1969, 1970), but the time lag from the first application to visible strobili varied with species, from 25 days in *Cupressus lusitanica* to 120 days in *Thuja plicata*. Different GAs also showed differential effectiveness in inducing strobili, and it was noted that short days were favorable for megasporangiate strobili development at the top part of the seedlings of *Cupressus lusitanica* and *Thuja plicata* (Pharis and Morf, 1968). *Thuja plicata* (Pharis and Morf, 1970) responded to GA level and photoperiod as did *Cryptomeria japonica*. High GA concentrations and short days favored megasporangiate strobili induction. Several Cupressaceae and Taxodiaceae needed a sequence of long day-short day-long day for development to maturity of strobili (Pharis and Morf, 1967, 1969). *Cupressus arizonica* strobili developed fully under short days but a sequence of short day-long day hastens the development compared to short days alone (Pharis et al., 1968).

These studies did not explain why GA failed to promote vegetative growth on *Cupressus arizonica*, as it did with *Cryptomeria japonica* and angiosperms. GA added to *Cupressus arizonica*, with AMO-1618, an ethylene producing compound which interferes with GA metabolism (Pharis et al., 1965b), did not alter strobili production but reduced growth response. By pinching off buds above microsporangiate strobili, and spraying the strobili with urea and GA, or urea and auxin, there was a transition to megasporangiate strobili in *Cryptomeria japonica* (Hashizume, 1961c).

Hashizume (1961d) examined the endogenous changes of auxin, nitrogen and carbohydrates in GA-induced shoots of *Cryptomeria japonica*. A pattern was found in which an auxin fraction decreased at induction time, and later increased gradually with maturation of the strobili, another auxin fraction appeared in high concentration at induction time. Total nitrogen decreased, and soluble carbohydrates increased with strobilus maturation. Later auxin studies (Hashizume, 1969) demonstrated the same trends in normally flowering *Pinus densiflora*. The endogenous GAs remained the same as in nonflowering trees at induction time. In megasporangiate buds the increase in a new auxin fraction was sharper than in microsporangiate buds. A growth inhibitor in the extracts from buds also increased with greater megasporangiate production in pines.

These studies support Lang's suggestion (Lang, 1965) that the adult phase at which trees enter the reproductive stage indicates the existence of an availability of hormones, or balance of hormones favorable to flowering. Addition of GA in certain Taxodiaceae and Cupressaceae seems to bring this balance into effect. Further, it seems likely that the two different kinds of reproductive structures require a different balance of growth substances and probably nutrients.

The present studies were designed to extend the observations on the influence of GA and interactions of fertilizers on flowering and growth in *Cupressus arizonica* seedlings. Megasporangiate and microsporangiate strobili were induced experimentally and their influence on vegetative growth tested. Interactions between GA, auxin and urea on growth and reproduction were followed. A study on the effect of local GA applications on different parts of the *Cupressus arizonica* seedling was undertaken to determine the movement of the flowering stimulus, and its influence on growth and reproduction. To obtain further insights into the endogenous metabolic changes and its control, studies were made on enzyme profiles in vegetative noninduced plants, as well as seedlings in transition to flowering.

# Chapter II

## INFLUENCE OF FERTILIZERS AND GA ON FLOWERING AND GROWTH

### Introduction

Studies indicate that GA induces strobili production, but does not promote shoot growth in Cupressus arizonica (Pharis et al., 1965a; Pharis and Owens, 1966; Pharis et al., 1967). This species is thus an ideal material in which to study growth suppression and metabolic changes associated with the transition from vegetative to reproductive stages. A similar effect also was observed in Thuja occidentalis (Fraser, 1970) where GA application caused growth to cease completely before strobili development started. Such observations suggest that control of vegetative and reproductive growth are closely interrelated

Nutrient status of trees and carbohydrate content influence growth and are important for reproductive development. Fertilization with nitrogen, phosphates or a balanced fertilizer mixture increased cone production in many conifers (Ebell and McMullen, 1970, Hoekstra and Mergen, 1957; Hausser, 1960; Wenger, 1953, Hansen, 1965, Hocker, 1962). The tallest and most vigorous trees, in good nutritional state produce the best cone and seed crops (Bramlett, 1971). In long day plants, added sugars promote flowering, while in short day plants added nitrogen promotes flowering (Chailakhyan, 1968) Nutrients are important in conelet and cone development. During the first and second year of development in pine, the cones act as channelling sinks for macro- and micronutrients from other parts of the plant (Dickmann and Kozlowski, 1969a)

Only at maturity do some macronutrients move out of the cone. Organic matter also accumulates in high amounts in developing cones (Dickmann and Kozlowski, 1970). In the first year of pine cone development, carbohydrates make up half of the weight of the conelet, and the cells are highly lignified, in the second year most reserve materials are used in cellulose production (Dickmann and Kozlowski, 1969b).

Els et al. (1965) compared annual cone production in *Pseudotsuga menziesii*, *Abies grandis* and *Pinus monticola* over a 28-year period to the annual ring width variation. They found that in years of high cone production rings were smaller in diameter than in those years of low production.

Optimal levels of carbohydrates have been correlated with flowering in several studies (Sachs and Hackett, 1969; Cumming, 1967). By girdling and studying carbohydrate levels at cone initiation in *Pseudotsuga menziesii* (Ebell, 1971), it was seen that starch content increased in the buds at strobili initiation time, with onset of cone development the amount of available carbohydrates and growth was reduced as was the frequency of new shoots and number of buds per shoot. For optimum strobili initiation, the best time for girdling preceded strobili initiation time (Ebell, 1971).

In broccoli (Fontes and Ozbun, 1972) soluble carbohydrates and starch increased at flowering time and GA applications increased starch content and flowering. In carnations, assimilates increased at the site of GA treatments (Harris et al., 1969). In *Cryptomeria japonica*, soluble carbohydrates increased after GA applications, while nitrogen decreased in differentiating buds (Hashizume, 1961d). One known action of GA on vegetative plant parts is that it increases electrolyte permeability (Artamonova and Artamonov, 1971), thus allowing for a higher flow of assimilates.

Different GAs have different affects on plants. In *Myosotis alpestris*, gibberellic acid ($GA_3$) promoted stem formation while $GA_7$ promoted stem formation and flowering (Michniewicz and Lang, 1962). In conifers a similar action is envisioned, since different GAs are differentially effective in flower initiation (Pharis and Morf, 1968). In *Cupressus arizonica*, four known GAs and two unidentified ones have been found (Ruddat et al., 1968). Probably different GAs have different functions; also, one kind of GA supplied to the plant may in some cases be converted to another one and regulate separate processes.

Experiments were undertaken to help understand the relationship between vegetative versus reproductive development in relation to fertilizers and GA application in *Cupressus arizonica* seedlings.

## *Materials and Methods*

Eighty individually potted two-year-old *Cupressus arizonica* seedlings were used. The seedlings were of unknown genetic stock from seeds obtained in 1969 from Dr. Pharis. The plants were divided into four groups of twenty seedlings and placed in a greenhouse. Starting May, 1971, each of the following treatments was applied to each tree in a group:

a) Fertilized once a week with 50 cc complete Hoagland's solution (Hoagland and Arnon, 1938),

b) Fertilized once a week with 50 cc complete Hoagland's nutrient solution and sprayed once weekly with 100 ppm $GA_3$ in aqueous .05% Tween-20 solution,

c) Sprayed once weekly with 100 ppm $GA_3$

in aqueous .05% Tween-20 solution, and

d) Control sprayed with aqueous .05% Tween-20 solution.

Measurements on growth were taken every second week for ten weeks. At the end of this period, strobili were counted and recorded.

## Results

The effect of fertilizer and GA applications on height growth are illustrated in Figure 1. GA applications and added nutrients increased seedling growth, compared to control. Both treatments seemed to have a synergistic effect on growth. Figure 2 depicts typical growth observed in the seedlings.

At the termination of this experiment, only microsporangiate strobili were detected and these only developed on plants subjected to GA treatments. Total number of strobili was greater on plants receiving fertilization than on those which received GA alone (Figure 3). Within each treatment, there were plants which did not produce strobili. The percentages of those which did not initiate reproductive development are shown on Figure 4.

Under the conditions of this experiment, the plants responded to GA treatment with strobili induction and height increment. Within the GA treated groups, some plants did not initiate reproductive development. Figure 5 shows the average growth of plants with strobili against those without. Analyzing growth of seedlings grouped into plants with more, and plants with fewer than fifty strobili each, a similar pattern emerged (Figure 6).

When growth of the three plants having the highest number of strobili were compared to growth of plants which did not produce strobili (Figure 7) differences were detected.

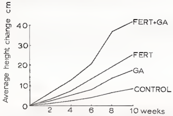

FIG1 Height growth response of *Cupressus arizonica*

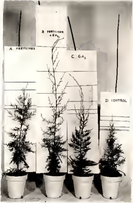

FIG 2 Height growth of Cupressus arizonica

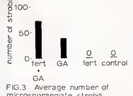

FIG.3 Average number of microsporangiate strobili

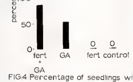

FIG.4 Percentage of seedlings with microsporangiate strobili

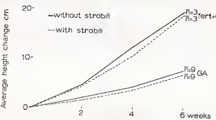

FIG 5 Height growth of seedlings with and without strobili

Improved nutritional status did result in a much higher strobili yield, but when average flowering and nonflowering plants were compared within a treatment, the difference in height was negligible.

## Discussion

The high growth rate of seedlings sprayed with GA compared to those not treated with GA was different than the findings of Pharis' group (Pharis et al., 1965a, Faris and Owens, 1966, Pharis et al., 1967). One possible explanation is that the temperature used in the previous experiments in Canada was lower (Pharis et al., 1965b), than that in the present experiments. Their experiments were done under 19°C day temperature and 15°C night temperature under 16-hour days with supplemental artificial light. The present experiments were run under summer conditions in Florida in a greenhouse without additional light. Their seedlings were less than one-year-old, while these were two-year-old. These factors may all have contributed to the difference in growth rates. In the present study height response to GA treatment was increased by fertilizer applications.

A channelling of nutrients presumably takes place at the time of strobili initiation. Control of the direction of this nutrient movement is desirable and possible with GA applications which regulate development. The differences in channelling nutrients are probably negligible under conditions of these experiments. Strobili growth was repprted to suppress vegetative growth rate in three conifers (Els et al., 1965). The strobili examined were mature and seed-filled.

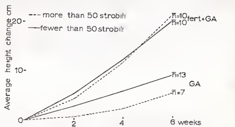

FIG 6 Height growth of seedlings with more and fewer than 50 strobili

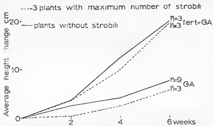

FIG 7 Height growth of seedlings with maximum number and without strobili

In the present case, only microsporangiate strobili were observed and the growth depression was negligible. If megasporangiate strobili had been induced, a stronger suppression of growth might have been found. This possibility is strengthened by the fact that heavy strobili-bearing seedlings had a lower growth rate than nonflowering ones in the same treatment.

An unexpected finding was the relatively low percentage of seedlings flowering (Figure 4) (85% in GA + fert. treatment, and 52.6% in GA treatment). Fertilization raised this fraction but still did not induce all to flower. Possibly some seedlings absorb less GA through their foliage than others. Appearance of the plants was quite variable, both in color and in size of leaf scales and growth habit. The phenotypic variation reflect a genotypic one, and this may also extend to the response to GA.

## Conclusion

Under the conditions of this experiment, i.e. the local photoperiod and Florida temperature in spring and summer, seedlings of *Cupressus arizonica* respond to GA by increased growth and development of reproductive structures.

Since only microsporangiate strobili were obtained in these experiments, a new approach was desirable, one in which megasporangiate strobili would be produced. Being larger than microsporangiate strobili, and growing for a longer time on the plants, megasporangia divert more energy from vegetative development, possibly reducing or influencing the growth rate more strongly than microsporangiate strobili. The above approach in young *Cupressus arizonica* is not directly comparable to studies of vegetative versus reproductive development made on large, mature conifers. However, it does suggest that the channelling of substrates during growth probably is interrelated, and that induction and differentiation are dependent on nutrient supply.

# Chapter III

## UREA, AUXIN AND GA EFFECT ON THE GROWTH AND SEXUALITY OF STROBILI

### Introduction

Induction and control of megasporangiate development in conifers is desired for regulating seed production. *Cupressus arizonica* seedlings particularly are convenient for studies of this regulation since they respond with strobili production after GA application (Pharis et al., 1965a). Moreover, in Cupressaceae, short day treatments and high GA concentration promote megasporangiate development (Pharis and Morf, 1966, Pharis et al., 1968), while long days and low levels of GA promote microsporangiate development (Pharis et al., 1970).

Cupressaceae and Taxodiaceae have a similar control mechanism. Short days also promote megasporangia development in *Thuja plicata* (Pharis and Morf, 1970; Owens and Pharis, 1971) and *Cryptomeria japonica* (Hashizume, 1959). Nitrogen supply and auxin content or supply have also been implicated with megasporangia development in many conifers. Nitrogen fertilizing favors flowering of long day species but inhibits flowering of short day species (Chailakhyan, 1968). Conifers have a slight difference in photoperiodic induction of the megasporangiate and microsporangiate strobili, microsporangiate strobili are differentiated earlier in summer than megasporangiate strobili. This suggests that the nitrogen effect on short day species primarily influences megasporangiate strobili differentiation in conifers.

In pines, nitrogen fertilization (Stephens, 1961) or nitrogen and phosphorus fertilization favored the transition of microsporangiate strobili to megasporangiate strobili production (Hashizume, 1961b). In *Pinus radiata* (Sweet and Will, 1965) nitrogen and phosphorus fertilizers almost eliminated microsporangiate strobili, but not megasporangiate strobili. In *Cryptomeria japonica*, GA and 1% urea sprays promoted megasporangiate development resulting in good seed production (Hashizume, 1960). In this species, treatment with 10,000 ppm urea, auxins and GA caused transition of microsporangiate primordia to megasporangiate strobili (Hashizume, 1961c).

Auxins have also been associated with a tendency toward femaleness in many plants, including conifers. In *Abies sibirica*, conelets form on the adaxial side of the shoots, where there is a higher auxin concentration than on the abaxial side, where microsporangiate strobili are formed (Minina and Balyaev, 1972). This difference in growth regulator activity is intensified in the branch at the time of strobili induction. At strobili induction, an endogenous auxin fraction increases in *Pinus densiflora* and *Cryptomeria japonica*. If shoots which normally bear microsporangiate strobili at induction time are pinched off above the developing buds these strobili will develop into megasporangia (Hashizume, 1969). This same transition affect can be achieved by auxin treatment in *Cryptomeria japonica* (Hashizume, 1961c). In pines, addition of 2, 4-D is unfavorable for microsporangiate strobili development (Brown and Greene, 1961; Hong, 1963).

Endogenous auxin content also shows a decreasing concentration gradient from the top of the plants down. In conifers this auxin gradient is reflected in the distribution of megasporangiate strobili on the upper parts (Pharis and Morf, 1967, 1968). Moreover, as a tree grows taller, strobili-bearing branches which were first induced to become megasporangiate, gradually become situated farther away from the auxin source and undergo a transition to bear microsporangiate strobili (Hashizume, 1961c).

If this hypothesis is correct, then to produce more megasporangiate strobili, one should treat *Cupressus arizonica* seedlings under a decreasing photoperiod, with a high urea fertilization and supply an external source of auxin. An alternate step would be to try to induce megasporangiate strobili at the sites where endogenous auxins occur in high concentrations, i.e. on the top part of the two-year-old seedlings.

Experiments were designed to test induction of megasporangiate strobili. Tests included photoperiod, urea, auxin and GA in different combinations

## Materials and Methods

Six-month-old *Cupressus arizonica* seedlings of commercial stock from the Andrews State Nursery at Chiefland, Florida, were used. Eighty individually-potted seedlings, divided into eight groups of ten each, were placed under 8-hour daylength, with a 25°C day and 20°C night temperature.

Sprays were applied three times per week as follows: $GA_3$ at 1000 ppm; IAA at 10 ppm, and urea at 10,000 ppm. Treatments were:

a) $GA_3$ + IAA + urea

b) $GA_3$ + IAA

c) $GA_3$

d) $GA_3$ + urea

e) IAA + urea

f) Urea

g) IAA

h) Control

All spray solutions contained .05% Tween-20 as a wetting agent; control received only the Tween-20 sprays. The experiment ran from October 31 to December 31, 1971. Height measurements were taken at the beginning and at the end of the eight-week period, strobili were counted at the end. All seedlings were watered with a 1/10 concentration of Hoagland's solution whenever necessary, i.e. about twice a week.

T tests were applied to the data to detect differences among the treatments in height, production of microsporangiate strobili and megasporangiate strobili (Steel and Torrie, 1960).

## Results

Influence of the different sprays on growth of the seedlings is presented in Figure 8.

Mortality was high where GA and urea treatments were combined, leaving only four actively growing seedlings for the final evaluation of treatments (a) $GA_3$ + IAA + urea and (d) $GA_3$ and urea. The effect of such a high concentration of urea was pronounced on seedlings elongating rapidly under GA influence. There was no mortality in the other treatments, and those surviving in treatments (a) and (d) were healthy.

Treatment effect on the number of microsporangiate strobili produced is shown on Figure 9.

Megasporangiate strobili were much fewer in numbers than microsporangiate strobili. No significant difference was found here among the treatments, due to the high variation of response found within each treatment. Figure 10 shows the results of megasporangiate strobili induction.

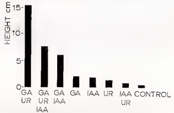

FIG 8 Height growth of 6 months old *Cupressus arizonica*

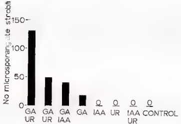

FIG 9 Average number of microsporangiate strobili

## Discussion

Growth promotion by any chemical growth factor tested here in spray (IAA, urea, GA) was better than control. Possibly nitrogen was a limiting factor to reproduction and growth. These data are strengthened by the fact that simultaneous application of GA and urea gave the highest growth rate, although it does not explain why the GA, urea and auxin treatment was so low.

For microsporangiate strobili induction it was not expected, from previous reports, that auxin would be effective. These results confirmed that observation made on other species than *Cupressus arizonica* (Hashizume, 1961c). Contrary to the expectation of nitrogen action on microsporangiate strobili development, GA and urea were the most effective treatment. This suggests that nitrogen may have been a limiting factor.

In megasporangiate strobili induction, auxin, urea and GA were no better than urea and GA treatments.

To test if nitrogen is limiting, a treatment with a fertilizer high in nitrogen would be expected to be a better substitute for urea, and one with a complete fertilizer mix but low in nitrogen, would be more beneficial than the no-urea treatments. Hashizume (1960, 1961c) successfully used this same urea concentration (1%) on *Cryptomeria japonica* but combined this with a 100 ppm GA concentration. In this study 1000 ppm GA which was used in conjunction with 1% urea resulted in a 40% survival rate of the seedlings, making statistical evaluations more difficult. These experiments with *Cupressus arizonica* should be repeated with lower concentrations of GA and urea. The 1000

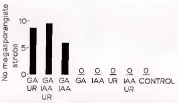

FIG10 Average number of megasporiate strobili

ppm is the usual rate for induction in Cupressus, possibly a synergistic effect occurs so that induction can occur at a lower concentration when urea is added.

### Conclusion

These experiments confirmed that high GA concentrations are effective in the promotion of megasporangiate strobili. However, 10,000 ppm urea solution is too high for application with 1000 ppm $GA_3$ on actively growing plants. Alternative research, in addition to reducing the concentrations applied to influence megasporangiate induction, might include treatments with different growth hormones, and GA applied at different locations on the plant.

# Chapter IV

# FLOWERING AND GROWTH OF *CUPRESSUS ARIZONICA* SEEDLINGS AS AFFECTED BY LOCAL APPLICATION OF GA

## Introduction

Flowering *Cupressus arizonica* seedlings have the general pattern of strobili distribution as other monoecious conifers megasporangiate strobili develop on the upper part of the tree (Pharis and Morf, 1968); microsporangiate strobili develop on the lower branches. This distinct distribution pattern makes *Cupressus arizonica* suitable for studies of the influence of local application of strobili inducing chemicals on flowering and growth.

The apical bud is a primary source of auxin production which moves in a basipetal gradient causing, among other things, a strong influence on lateral buds and branch growth, i.e. apical dominance. In the area of high auxin concentration, megasporangiate strobili develop, suggesting a correlation between high auxin and femaleness (Pharis and Morf, 1967, 1968). This hypothesis is supported by the observations that 1) auxin is associated with megasporangiate development in *Cryptomeria japonica* (Hashizume, 1961c), 2) auxins increase at the time of strobili induction in *Pinus densiflora* and *Cryptomeria japonica* and this increase is stronger with megasporangiate strobili induction than microsporangiate induction in *Pinus densiflora* (Hashizume, 1969); 3) auxin is always associated with megasporangiate strobili induction and development in *Abies sibirica* but not with microsporangiate strobili (Minina and Belyaev, 1972); and 4) GA treated species of *Cupressus* and *Thuja* elongate very rapidly, and megasporangiate strobili are borne at distances further away from the tip and the branches undergo a redifferentiation, to produce microsporangiate strobili.

Microsporangiate strobili can be induced in *Cupressus arizonica* with GA sprays (Pharis et al., 1965a). Long days result in microsporangiate development (Pharis et al., 1970) as does application of low concentrations of GA. Induction of megasporangiate strobili requires short days (Pharis and Morf, 1968, Pharis et al., 1968) and high concentrations of GA. *Cryptomeria japonica* (Hashizume, 1959) responds in the same manner. However, even under conditions favorable to megasporangiate development, the number of buds developing into microsporangiate strobili is much greater.

Movement of GA has been reported in $^{14}$C-tracer studies (McComb, 1964). In corn and bean plants, there was first an acropetal movement, followed by a distribution over the whole plant of the $^{14}$C-label applied to the middle of the plant (Zweig et al., 1961). In soybean (Crafts, 1967a, b) 2, 4-D-$^{14}$C applied to the lower part of the plant moved basipetally, application to the top part, moved acropetally. If 2, 4-D was applied to centrally located leaves, however, it moved both ways. If *Cupressus arizonica* has a similar pattern for GA movement, local control of induction of megasporangiate and microsporangiate strobili might be accomplished. Thus, for megasporangiate development it would be ideal if only the top part of the seedling could be induced, while only lower branches could be induced to form microsporangiate strobili when they are desired. A method of local, limited GA application to one part of a stem as a possible way to manipulate cone induction was tested.

Experiments were designed to determine the interaction of photoperiod with strobili induction if only part of the seedlings were sprayed, and how concentration of GA applied to the seedlings affect growth and the kind of strobili induced.

## Materials and Methods

Seventy-two nine-month-old individually potted seedlings were used, selected as evenly as possible for size and the same physiological condition. The seedlings were of commercial stock, unknown genetic origin, obtained from Andrews State Nursery at Chiefland, Florida. One group of 36 seedlings was placed under 8 hour light, the other under 16 hour light; temperature of light period was 25°C and of dark period was 20°C. Each group was divided into nine treatments of four plants each. Seedlings were grown two months under the photo-regimes before treatments were started. All seedlings were watered at least twice weekly with 1/10 strength Hoagland's (Hoagland and Arnon, 1938) solution. Height was recorded weekly. After eight weeks of treatment, strobili were counted on each 1/3 section, i.e., upper, middle, and lower part of the seedling.

Treatment solutions were applied to seedlings in an aqueous .05% Tween-20 solution and applied twice weekly as follows:

a) 100 ppm $GA_3$ on the whole seedling,
b) 100 ppm $GA_3$ on lower 1/3 of seedling,
c) 100 ppm $GA_3$ on middle 1/3 of seedling,
d) 100 ppm $GA_3$ on upper 1/3 of seedling,
e) 1000 ppm $GA_3$ on the whole seedling,
f) 1000 ppm $GA_3$ on lower 1/3 of seedling,
g) 1000 ppm $GA_3$ on middle 1/3 of seedling;

h) 1000 ppm $GA_3$ on upper 1/3 of seedling, and,

i) Control (only Tween-20 sprays).

## Results

The influence of the different photoperiods and concentrations of GA on height is shown on Figure 11. Under both 100 and 1000 ppm GA, the 16 hour photoperiod had a marked influence on growth, resulting in an increase of more than 100% over the growth increase under 8 hour photoperiod, e.g. 20 versus 46 cm. This height increase, also noted in the control plants, is greatly enhanced by GA. In the 1000 ppm treatments, the growth rate of the seedlings receiving local sprays approached the growth rate of the seedlings receiving sprays on the total plant. Those seedlings receiving local sprays of 100 ppm GA, grew at a rate in between those of total seedling spray and the controls. At 100 ppm, it was more effective to spray middle and lower branches than upper for growth.

The average number of strobili produced per seedling per photoperiod and GA concentration, regardless of the part of the seedling sprayed, is presented in Figure 12. Under 16 hour days, the effect of GA concentration on kind of strobili induced is more easily assessed. GA at 1000 ppm leads to relatively greater increases in megasporangiate than microsporangiate strobili. A 100 ppm GA concentration induces relatively more microsporangiate than megasporangiate strobili. Under a short photoperiod the concentration effect is not so evident, probably because the total numbers of strobili of both kinds induced is very low.

Figure 13 presents data on the number and kind of strobili induced on every part of the seedling after total and partial sprays of

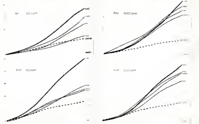

FIG11 Height growth with localized GA application(cm)

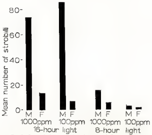

FIG 12 Strobili production of Cupressus arizonica

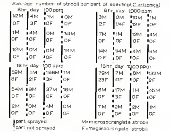

FIG13 Average number of strobili per seedling part

100 and 1000 ppm GA under 8 and 16 hours photoperiod. Controls, which did not receive $GA_3$, produced no strobili. Megasporangiate strobili developed on the top part of the seedling, with only one treatment overlapping into the middle 1/3, i.e. 16 hour day and 1000 ppm GA applications. Microsporangiate strobili developed all over the seedling.

Under 8 hour days, at both GA concentrations there was not much mobility of the megasporangiate and microsporangiate strobili inducing factor. A better distribution of megasporangiate strobili occurred on the upper part of the seedling, and microsporangiate strobili on the lower part, even under total spray. Under day length of 16 hours the direction of the strobili inducing factor had a high tendency to move up, both for microsporangiate strobili and megasporangiate strobili. The tendency for the stimulus to move upward was evident also for microsporangiate strobili, when seedlings sprayed on the lower and middle thirds in 16 hour photoperiod are examined. Under these conditions, spraying the top part of the seedling with either concentration resulted in a much higher megasporangiate strobili production than microsporangiate in the sprayed region.

There was some movement of the strobili inducing factor downwards, in particular when the concentration of GA applied was high (1000 ppm), but not as much as the upward mobility. Under 8 hour days, downward movement was very low compared to observations at 16 hours.

## Discussion

Photoperiod greatly influences height of _Cupressus arizonica_ seedlings. A greater height growth response results from a 16 hour photoperiod treatment than an 8 hour photoperiod. Influence of GA on height growth is not as great as photoperiod, but 1000 ppm sprays on total seedlings enhances growth 100% more than 100 ppm under short days. Under long days growth rate is a little lower on total sprays at 1000 ppm than at 100, possibly because of some GA toxicity. This conclusion is strengthened by the fact that partial 1000 ppm sprays at 16 hours have a higher affect on growth than partial 100 ppm sprays at 16 hours. High GA concentration and long photoperiod increase overall number of vegatative and reproductive buds, but the ratio of vegetative versus reproductive buds remains the same (Pharis et al., 1970). However, high GA concentration and long photoperiod treatments yield more megasporangiate strobili than low GA concentration and short photoperiod. This affect may be related to auxin. Apical dominance has a stronger expression under inductive conditions (Pharis, 1965b). Auxin may also induce ethylene production, which also increases induction of megasporangiate strobili (Bonnet-Masimbert, 1970) in Cupressus arizonica seedlings.

The flowering stimulus induced by GA application has a stronger tendency to move upwards than downwards, and this tendency is enhanced by long photoperiod. Some control of the kind of strobili induced is possible, as evidenced by the following experimental observations. Spraying the upper top of the seedling results in a much higher number of megasporangiate strobili, especially on the top, than microsporangiate strobili. Spraying the lower part of the seedling will result almost exclusively in induction of microsporangiate strobili, and due to the upward mobility of the stimulus, they will appear in higher numbers on the top than on the lower part, especially under long days.

Another interesting growth characteristic in Cupressus arizonica is that the strobili inducing stimulus has distinct expression on the top of the seedling, depending on where GA was first applied. GA applied to the top of the seedlings results in megasporangiate strobili on the top. Several interpretations are possible. One, the site where GA penetrates the plant may be crucial: GA interaction with lower branches could allow only for expression of microsporangiate strobili, whereas, GA entering and interacting with upper branches allows for the induction primarily of megasporangiate strobili. Two, the stimulus for the production of different strobili could be different kinds of GA. Alternatively, GAs applied to the top may find a metabolic state, e.g. information in mRNA, limited to inducing megasporangiate strobili. In this context, it has been shown that if GA is applied to the top of a plant, carbohydrates move to this area, if applied to the lower part, carbohydrates move down, if applied to the middle, carbohydrates move both ways (Zweig et al., 1961, McComb, 1964). Thus, the carbon pool to form new cells is flexible, what type of new cells are induced is probably precoded before GA initiates the growth.

A paper published after this study was concluded (Pharis et al., 1972) shows that GAs applied centrally to a *Cupressus arizonica* seedling, move both ways, but during the first 24 hours moved mainly upwards. This is in agreement with the findings here that the flowering stimulus moves acropetally. Since seedlings were repeatedly sprayed in my experiments the opportunity existed for repeated upward movement of GA, with the mobilization of carbohydrates essential for growth being more available in seedlings exposed to 16 hour photoperiod than in seedlings exposed to the 8 hour photoperiod.

## Conclusion

It is possible to influence and regulate development of the kind of strobili desired in Cupressus arizonica. Under 1000 ppm GA and a long 16 hour photoperiod, plants sprayed only at the top produced mainly megasporangiate strobili, those sprayed at the lower branches produced mainly microsporangiate strobili, and these are concentrated at the upper portions of the plant. Plants with mainly megasporangiate development showed a growth suppression when compared with those plants having mainly microsporangiate development.

Refinements of this technique with intra-stem feeding of GA to the exact location desired, and applications of auxins and nutrients or modified photoperiods are promising possibilities for the control of strobili development.

## Chapter V

# ENZYME STUDIES IN *CUPRESSUS ARIZONICA* AFTER GA APPLICATIONS

## Introduction

*Cupressus arizonica* seedlings can be induced to flower by application of GA (Pharis et al., 1965a). The induced morphological changes are associated with physiological changes regulated by enzymes. *Cupressus arizonica* treated with GA provides good material to study the enzyme processes associated with flowering.

Growth substances act on plants in many ways. One way includes production of new RNA (Key, 1964, Osborne, 1965). Flowering has been related to new RNA synthesis in many plants (Salisbury, 1963; Searle, 1965; Kupila-Ahvenniemi and Pahakasi, 1966, Bernier, 1971). Flower induction and development is also associated with new proteins in *Pharbitis nil*, *Tulipa*, *Geum urbanum* and *Acalypha ornata* (Marushige and Marushige, 1962, Barber and Steward, 1968, Tran and Trippl, 1969, Kahlem, 1970). Van Overbeek (1966) postulated that GA acts on the genes, increasing production of hydrolytic and proteolytic enzymes in cells; the enzyme changes increase auxin content and the induced cells generally act as nutrient sinks. GA may also repress IAA oxidases with the increased auxin leading to various physiological changes (Kögl and Elema, 1960; Galston and McCune, 1961). GA has been reported to increase auxin production and diffusion in plant tissues (Valdovinos et al., 1967) and to alter overall enzyme activity while inducing flowering in *Trifolium pratense* ([illegible] and Stoddart, 1970). These results suggest that the primary action of GA may be to induce protein synthesis in the shoots.

Flowering in woody species normally occurs after attainment of the adult stage. Aging has been reported as being accompanied by increases of peroxidase bands (Ockerse et al., 1966). In *Robinia pseudoacacia*, α-amylase, polyphenol oxidase and peroxidase increase in transition from juvenile to adult phase, polyphenol oxidase decreases with senility.

Hormones directly or indirectly cause changes in enzyme activity or isozymes. Ethrel, a source of ethylene, increased peroxidase activity in cucumber (Retig and Rudich, 1972) and decreased IAA oxidase activity, concomitant with higher auxin levels and femaleness. IAA caused an increase in peroxidase activity in *Pelargonium* tissue culture (Lavee and Galston, 1968). Ethylene also increased peroxidase activity in sweet potato discs, and two new peroxidase bands appeared, the ethylene effect was prevented by cycloheximide and actinomycin D. This suggests that ethylene acts through transcription and translation (Gahagan et al., 1968). Isoperoxidase patterns are altered by addition of IAA or GA to the plants (Street and Cockburn, 1972). In conifers, peroxidase has been associated with high mitotic index (Lebedenko, 1967), which may be reflecting a high metabolic rate accompanying cell division.

Synthesis of α-amylases in barley endosperm tissue is completely dependent on GA (Varner and Chandra, 1964; Varner, 1964). Addition of $GA_3$ resulted in two new isozymes in this tissue (Momotani and Kato, 1966, Tanaka and Akazawa, 1970). In growing plants, α-amylase activity has been correlated with increased growth after GA treatment (Katsumi and Fukihara, 1969; Carter et al., 1973). GA could promote growth by supplying sugars from starch degradation.

Polyphenol oxidase (PPO) has also been activated by hormones. Ethylene increased the activity of amylase, peroxidase and polyphenol oxidase in cotton leaves (Herrero and Hall, 1960). In potato tubers and parsnip roots ethylene increased PPO but not peroxidase activity (Stahmann et al., 1966). Indole-acetic acid also stimulates PPO, apparently through the action of a phenolic component (Vernon and Straus, 1972). PPO also produces quinones, from which sugar-binding flavonoids are derived.

Phenylalanine ammonia-lyase (PAL) catalyses the conversion of phenylalanine to trans-cinnamic acid, a precursor of lignin. Plants with high rates of lignin synthesis have high PAL activity. In several plant species GA was reported to indirectly stimulate PAL activity (Reid and Marsh, 1969). GA induced PAL activity and growth were not proportional in dwarf peas (Cheng and Marsh, 1968). In tobacco tissue culture, NAA and GA in growth media, each decreased PAL activity; no effect on direct enzyme assay was found (Leonova and Gamburg, 1972).

No enzyme has been related specifically to flowering. But the flowering process seems to be associated with different enzyme activities than those in vegetative growth. This study was conducted to discern if certain enzyme changes could be detected with the transition to flowering in GA induced *Cupressus arizonica*. The following enzymes were compared: α-amylase, PAL, peroxidase and polyphenol oxidase.

## Materials and Methods

Two-year-old seedlings from commercial stock raised at the Andrews

State Nursery, Chiefland, Florida, were used. Eighty seedlings were individually potted and divided into four groups of twenty and placed in an unheated greenhouse at Gainesville under natural daylight conditions. These experiments ran from February 1 to March 30, 1973.

The plants were subjected to the following treatments:

a) Control

b) 1. Sprayed twice weekly with 0.5% Tween-20 and 1000 ppm $GA_3$
   2. Sprayed twice weekly with 0.5% Tween-20 and 1000 ppm $GA_3$
   3. Sprayed twice weekly with 0.5% Tween-20 and 1000 ppm $GA_3$

Branch material was collected from each plant on the following days: February 1, 8, 16, 20, 24, 28, March 4, 8, 12, 16, 30. Branches approximately 5 cm in length were taken from the upper half of the seedling, each containing between five and ten buds. Material from each group of plants was pooled and immediately dropped into liquid nitrogen. Acetone powders were prepared from each group of seedlings, and subsequently enzymes were extracted and submitted to gel electrophoresis. Acetone powders were made by grinding the material in liquid nitrogen and washing with acetone at -15°C to -20°C. The powders obtained were dissolved with the following protein extracting solution modified from Hare (1970):

| | |
|---|---|
| Urea | 15.0g |
| $K_2S_2O_5$ | 0.5g |
| Ascorbic acid | 1.0g |
| Cleland's reagent | 0.1g |
| Tween-20 | 4.0ml 10% |
| PEG 6000 | 15.0g |
| Tris to pH | 8.5 |

Double distilled water to 100 ml

Thirty microliters of the extractive were applied to polyacrylamide gels with spacer gels, and run for 50 minutes at 3 milliamperes per gel, with a pH 8.9 tris buffer in the upper chamber, and pH 8.1 tris buffer in the lower chamber. Later the gels were submitted to enzyme substrate and staining. PAL activity was measured as described by Zucker (1965). Acetone powders were dissolved in 0.1 M borate buffer pH 8.8, and the solution supplied with 60 micromoles of L-phenylalanine as substrate. Formation of trans-cinnamic acid was measured at 30 minutes time intervals for two hours on the spectrophotometer at 290 nanometers.

To test for environmental influences, a new experiment was set up, with two groups of plants under 12-hour days with night and day temperature at 15°C and 20°C. One group was sprayed twice weekly with 1000 ppm GA. Two other groups were placed under 12 hour days and day temperatures at 25°C and 30°C, with one group sprayed with 1000 ppm GA, and the other as control. Collections were made every five days for enzyme analysis.

## Results

Enzyme and protein data averages are summarized in Table 5-1. A few examples of typical bands obtained in the procedures used are shown in Figures 14 and 15. An actual photograph of gels as scanned is shown next to the representation for peroxidase (Figure 14).

### Peroxidase

Group A had 12 bands, $B_1$, B, $B_2$, B, and $B_3$, B. The pattern within each group was consistent with little variation from time to time. No response to GA treatment was apparent.

### Polyphenol Oxidase

All groups had 6 bands, varying in intensity according to group. No treatment effect was obtained.

### Amylase

From time 0 to 28 days, all groups, A, $B_1$, $B_2$, and $B_3$, showed one band, except group $B_2$ had 3 weak bands at the beginning of the experiment. On days 32, 36 and 40, there were from 3 to 4 bands visible. On days 44 and 58 there were 6 to 7 bands in all treatments. These results varied with time within each group and not according to treatment.

### PAL

Responses to PAL were highly variable within groups, treatments and with time. No clear isozyme pattern could be discerned.

### Total Protein

All 4 groups presented the same pattern, with 10 bands always clearly visible (Figure 14). Bands 7 and 8 had the same staining concentration throughout, except on day 44, when band 8 was much stronger than band 7, and on day 58 when band 7 was more intensely stained. This pattern of variation was also consistent within each group including control seedling buds.

### Environmental Influence

At day 60, no strobili were visible. Since no transition to strobili induction occurred, no enzyme analyses were performed.

## Discussion

The absence of treatment effect may have several origins.

1. Since 5 cm of shoot tissues with buds were removed for analysis, the amount of tissue extracted may be too large in comparison with the small buds, and cell masses where differentiation was taking place.

Table 5-1. Number of enzyme bands in _Cupressus arizonica_.

| | Days After Trmt. | Total Protein | | | | -Amylase | | | | Peroxidase | | | | Polyphenol Oxidase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | $B_1$ | $B_2$ | $B_3$ | A | $B_1$ | $B_2$ | $B_3$ | A | $B_1$ | $B_2$ | $B_3$ | A | $B_1$ | $B_2$ | $B_3$ |
| | 0 | 10 | 10 | 10 | 10 | 1 | 1 | 3 | 1 | 12 | 8 | 8 | 8 | 6 | 5 | 6 | 6 |
| | 8 | 10 | 10 | 10 | 10 | 1 | 1 | 1 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| | 16 | 10 | 10 | 10 | 10 | 1 | 1 | 1 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| | 20 | 10 | 10 | 10 | 10 | 1 | 1 | 1 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| | 24 | 10 | 10 | 10 | 10 | 1 | 1 | 1 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| | 28 | 10 | 10 | 10 | 10 | 1 | 1 | 1 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| Swelling of buds ($B_{1,2,3}$) | 32 | 10 | 10 | 10 | 10 | 3 | 4 | 4 | 4 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |
| | 36 | 10 | 10 | 10 | 10 | 3 | 4 | 4 | 4 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 5 |
| | 40 | 10 | 10 | 10 | 10 | 3 | 4 | 4 | 4 | 12 | 8 | 8 | 8 | 6 | 6 | 5 | 6 |
| | 44 | 10 | 10 | 10 | 10 | 6 | 7 | 7 | 7 | 12 | 8 | 8 | 8 | 6 | 6 | 5 | 6 |
| Strobili developed ($B_{1,2,3}$) | 58 | 10 | 10 | 10 | 10 | 2 | 2 | 2 | 1 | 12 | 8 | 8 | 8 | 6 | 6 | 6 | 6 |

A = Control

$B_{1,\ 2,\ 3}$ = 1000 ppm BA twice/week

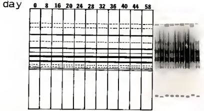

FIG14 Peroxidases in *Cupressus arizonica*(A)

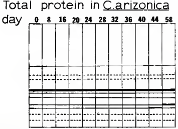

FIG.15 Total protein in Cupressus arizonica

2. There was no effect of GA or strobili induction on the limited enzymes studies. *Cupressus* and/or gymnosperm bud transition to flowering may be reflected by different enzyme activities than those commonly studied in angiosperms or in this study.

3. There were many branches on plants which did not flower within the group of 20 seedlings. The enzyme patterns of branches which did not show strobili induction masked those which did.

Variations detected (Table 5-1) in α-amylase and total protein patterns may have been due to environmental influences. After day 28, when the weather turned warmer, several isozymes appeared in all groups. Total protein bands were also different in the last two test dates, with the intensities of band 7 and 8 changing. Again, this was probably due to environmental influences since it occurred in all treatments and control.

The failure to produce strobili may be related to the photoperiod. In *Thuja occidentalis*, 12 hour light periods are the least affective for strobili induction (Fraser, 1970).

## Conclusion

In spite of the time spent on enzyme methods, results were not correspondingly fruitful. However, advancements were made in perfecting techniques and expected results from enzyme studies warrant further reserach efforts with still further improvements in methods.

These improvements should include the use of (1) large population of clonal plant material, (2) controlled conditions for induction, and (3) excised buds only instead of buds and subtending branch tissues. The improved techniques for extraction of enzymes in such small amounts of bud tissue have been recently reported (e.g. Haissig and Schipper, 1972). It would be particularly worthwhile to find out if amylase enzyme changes represent responses to temperature and what caused the changes in band intensity in the total protein fractions.

Testing for total activity, not just isozyme variations in such enzymes as amylase, IAA oxidase, peroxidase and polyphenol oxidase also may yield more answers relative to more answers relative to metabolic activity and growth changes.

## SUMMARY AND CONCLUSION

Very little is known about physiological changes occurring within a plant during transition to flowering. This work was undertaken in an attempt to learn more about floral induction and to perfect some techniques with the plant, Cupressus arizonica, which readily responds to chemical treatment of GA by producing strobili. Conclusions which can be drawn from the present study are:

1. $GA_3$ induces both vegetative and reproductive growth of Cupressus arizonica. Seedling response is varied, some produce strobili profusely, others scarcely and some none at all. This suggests that genetic material highly responsive to GA, or nonresponsive, can be clonally propagated and offers better future experimental material.

2. Complete fertilizer or urea application enhances response to $GA_3$. However, the combination of GA and high concentration of urea, is toxic to some Cupressus plants, but those which survive grow considerably taller, and produce both more megasporangiate strobili and more microsporangiate strobili than any other combination of urea, GA and auxin.

Presumably an auxin is involved in regulating reproductive development of growth of megasporangiate strobili. The effective concentration of auxin may be different from the one used here. It can be speculated that absorption of hormones through the outer leaves is different from plant to plant, corresponding to the variation in their phenotypic appearance, which shows a remarkable range in color and shape. A preliminary experiment with a limited number of seedlings showed that injection of $GA_3$ is very efficient to insure uptake.

3. Short photoperiods and application of high GA concentrations are conducive to a higher ratio of megasporangiate strobili to microsporangiate strobili than long photoperiods and applications of low GA concentrations.

4. An efficient method was developed to selectively produce either largely megasporangiate strobili or microsporangiate strobili on *Cupressus arizonica* seedlings: applications of GA to the top part of a plant result mainly in production of megasporangiate strobili, whereas, GA applications to the lower branches result almost exclusively in microsporangiate strobili being induced. This is in accord with the view that the control of the kind of strobili produced is dependent on a stimulus and the nutritional status of the plant part where strobili will be initiated. The flowering stimulus has a marked tendency to move upwards. This tendency, and the difference of response of applications to the lower and upper part of plants, result in the top of the plant producing megasporangiate if GA is applied to the top, but microsporangiate if application is on the lower parts. Thus, the upper branches respond differently depending on where the stimulus was first applied. Considering studies of GA movement in other plants, it is possible to link this difference in response to nutrient contents. GA applied to the lower parts drains carbohydrates down from the top resulting in the production of microsporangiate strobili on the upper branches; GA applied to the top moves carbohydrates up, and megasporangiate strobili are produced.

This hypothesis can easily be tested by following movement of $^3H$ labelled GA and $^{14}C$-labelled carbohydrates and content in the plant.

5. Enzyme extraction and detection in Cupressus arizonica tissues works well, providing relatively large amounts of plant material are available. But this is a handicap in detecting enzyme changes in the actual microscopical amounts of tissues where induction and transition to flower bud occurs. Improvements can probably be made in the techniques applied to micro amounts of protein.

6. Genetically homogenous material would be preferable to the variable source material used in these studies. The large range of variation among Cupressus arizonica seedlings is a disadvantage in physiological-biochemical studies. Clones can be established for uniformity. In this aspect Cryptomeria japonica may be more useful. It is a commercially more important species, is easily propagated vegetatively, and has larger buds. Cryptomeria japonica also produces strobili after GA treatment.

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# BIOGRAPHICAL SKETCH

Arno Brune was born October 22, 1941 in Bad Berneck, Germany His family returned to Brazil in 1946. In 1960 he graduated from high school in São Paulo. After serving in the Brazilian army in 1962, he studied forestry at the Universidade Federal de Vicosa from 1964 to 1967. In the first half of 1968 he worked as a forest geneticist in the Forest Service of São Paulo State. In August, 1970, after 2 years of study he received the master's degree in forest genetics at the University of Washington in Seattle. From 1970 to present, doctoral work was pursued at the University of Florida in forest physiology

Arno Brune is married to the former Lujan N. Chagas and has one daughter.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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